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(54) Title: NUTRITIONAL PRODUCTS CONTAINING ACID-TREATED MODIFIED STARCHES

(57) Abstract

Modified starch materials having a number average molecular weight of at least 10,000 for nutritional products provide a relatively slow release of metabolizable carbohydrates, giving a source of carbohydrate energy over a longer period of time than can be obtained from glucose and other carbohydrates such as lactose, fructose, or sucrose. Such modified starch material possess altered processing and pasting profile characteristics.

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NUTRITIONAL PRODUCTS CONTAINING ACID-TREATED MODIFIED STARCHES

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RELATED APPLICATIONS

This application is a continuation-in-part of Serial No. 08/948,677, filed October 10, 1997, which is a continuation of Serial No. 08/465,884, filed June 6, 1995, for "Nutritional Products Containing Modified Starches," now U.S. Patent No. 5,695,803, issued December 9, 1997.

FIELD OF THE INVENTION

This invention relates to nutritional products containing modified starches and, more particularly, to water-soluble modified starches and to nutritional products including such starches characterized by highly desirable properties.

20

BACKGROUND OF THE INVENTION

Carbohydrates are used as the major energy source in a variety of nutritional products ranging from, for example, liquid nutritional supplements for adults with compromised digestive functions, infant formulas, and carbohydrate supplements for exercise and athletic activity. Carbohydrate supplements are likewise included in a variety of solid nutritional products.

The carbohydrates previously used as such supplements generally comprise glucose (dextrose), fructose, lactose, sucrose, and glucose polymers. Such carbohydrates typically are of relatively low molecular weight.

The use of these previously used carbohydrate sources generally exert a degree of osmolarity that, in many situations, is undesirable. Thus, the use of such carbohydrate supplements or sources can result in consequent gastrointestinal effects such as diarrhea and

distention. In addition, the use of such carbohydrate sources can limit the availability of water for hydration purposes. Additionally, the high insulin responses to glucose polymers can lead acutely to elevated 5 postprandial insulin secretion and lipogenesis, hypoglycemia, and chronically to elevated blood lipids and increased risk for cardiovascular disease.

Accordingly, to serve as the major energy source for enteral dietary formulations, other liquid nutritional 10 supplements and other foods in general, the carbohydrate source must satisfy several criteria. From the physiological viewpoint, a suitable carbohydrate source should desirably have satisfactory glycemic, endocrine, and metabolic responses under varying physiological 15 conditions. Additionally, no significant adverse effects on body composition and lipogenesis should result from including the carbohydrate supplement. Still further, such carbohydrate sources must be capable of being adequately digested and, preferably, have appropriate 20 oxidation characteristics.

From the physical standpoint, the carbohydrate source should have sufficient water-solubility, particularly when used in liquid supplements so that useful amounts of the carbohydrate source can be utilized 25 in the formulation. It is also desirable for the carbohydrate source to be capable of being readily gelatinized.

Prior literature reflects substantial work relating to the effects of starches as nutritional supplements. 30 It has thus been suggested that the structure of food starches affects the rate of digestion of the starch as well as the glycemic and insulin responses after digestion. Behall et al., AEffect of Starch Structure on Glucose and Insulin Responses in Adults, Am. J. Clin. Nutr. 35 Nutr., 47, 428 (1988); Ross et al., AGlycemic Index of Processed Wheat Products, Am. J. Clin. Nutr., 46, 631 (1987).

A slow rate of starch digestion, giving low glycemic and insulin responses are likewise considered desirable for the general population, during sustained physical activity. Davis et al., "Carbohydrate-Electrolyte Drinks:

- 5 Effects on Endurance Cycling in the Heat," Am. J. Clin. Nutr., 48, 1023 (1988); Geske et al., "Preexercise Ingestion of Glucose and Fructose: Effects on Exercise Performance When Exercise is Begun at Predetermined Time of Peak Blood Concentration of the Sugar," Med. Sci. Sports, 16 190 (1984). Such a slow rate is also desirable for use in the diets for diabetic individuals. Crapo, "Simple Versus Complex Carbohydrate Use in the Diabetic Diet," Am. Rev. Nutr., 5, 95 (1985). Such a diet would lower the dependence of diabetics on deleterious
- 10 15 high fat diets by enabling more starchy foods with lower glycemic indices to be introduced into the diet.

It has been proposed that starches with high amylose content yield more sustained plasma glucose levels with lower requirements for insulin. Behall, et al. "Effect of Starch Structure on Glucose and Insulin Response in Adults," Am. J. Clin. Nutr., 47, 428 (1988). Likewise, it has been stated by Behall et al. that subsequent blood lipid and circulating insulin levels also may change after ingestion of high amylose starches.

- 20 25 Data has also shown that glucose and glucose polymers, fed simultaneously with calcium, can increase calcium absorption in healthy subjects and in hospitalized patients. Bei et al., "Glucose Polymer Increase Jejunal Calcium, Magnesium and Zinc Absorption in Humans," Am. J. Clin. Nutr., 44, 244 (1986); Knowles et al. "Response of Fractional Calcium Absorption in Women to Various Administered and Glucose Doses," Am. J. Clin. Nutr., 48, 1471 (1988). However, it has also been noted that diets high in simple sugar can increase calcium excretion in urine by the same mechanism as dietary protein and amino acids, i.e., inhibition of reabsorption
- - -

- of calcium in the kidney tubules. Inhibition of calcium reabsorption by insulin is at least partially involved. Holl et al., "Comparative Effects of Meals High in Protein, Sucrose, or Starch on Human Mineral Metabolism and Insulin Secretion," Am. J. Clin. Nutr., 48, 1219 (1988). Accordingly, it would be undesirable to employ carbohydrates that are rapidly metabolizable, ostensibly as an aid to calcium absorption, if such carbohydrates simultaneously increased calcium excretion.
- 10 However, the use of starches characterized by a low glycemic index might serve to increase calcium absorption, particularly in the jejunum, ileum and large intestine, unaccompanied by the insulinogenic effects of glucose or the like, which block calcium reabsorption.
- 15 Starches of this type might be preferred over glucose polymers in dietary products for physiological conditions where assurance of sufficient energy intake is needed and where maintenance of body calcium is most important. Conditions such as osteoporosis, diabetes, prolonged
- 20 physical work, or even prolonged missions in gravity-free space are illustrative examples.

Further, the use of carbohydrates as a major energy source in a nutritional supplement pose a major problem. Typically, these carbohydrates exert a degree of

25 osmolarity that is undesirable in many situations because of the subsequent gastrointestinal effects such as diarrhea or distention. Presently used glucose polymers, consisting of mixtures of relatively small molecular weight molecules, disaccharides and monosaccharides, do

30 decrease the osmotic pressure somewhat in comparison to other carbohydrate sources. However, the decrease in osmotic pressure which results is generally not as much as is desired, nor is the decrease obtained very reproducible.

35 Still further, it would be desirable to provide starches having processing characteristic tailored to satisfy the requirements for particular applications.

- More specifically, sometimes it is desired to utilize starches achieving gelling at a lower temperature and requiring reduced energy. Other applications utilize retrograded starch, and it would be desirable to select
- 5 retrograded starches having satisfactory gelling characteristics. In still other applications, it is desirable to enhance the pasting properties of starches, e.g., so as to reduce the paste viscosity and facilitate processing.
- 10 Additionally, one major obstacle to the use of starches for many applications is their typically characteristic water insolubility. Relatively water insoluble materials would be unsuitable for many nutritional supplements such as, for example, enteral
- 15 dietary formulations and liquid nutritional supplements.
- Chemical modification of starches by various techniques is also well known. For example, acid treating of starch was explored late in the 19th century. Some properties of these acid-treated starches, as
- 20 compared to those of the untreated starches, include decreased intrinsic viscosity and decreased gel strength.
- Also, Ma and Robyt, "Preparation and Characterization of Soluble Starches Having Different Molecular Size and Composition, by Acid Hydrolysis in Different Alcohols,"
- 25 Carbohydrate Res., 166, 283 (1987), reported modifying potato and waxy maize starches with hydrochloric acid in methanol, ethanol, 2-propanol and 1-butanol at 65°C for one hour. The modified starches were readily soluble in hot water, giving crystal-clear solutions up to a
- 30 concentration of at least 20% (w/v).

More recently, Fox and Robyt, "Modification of Starch Granules by Hydrolysis with Hydrochloric Acid in Various Alcohols, and the Formation of New Kinds of Limit Dextrins," Carbohydrate Res., 227, 163 (1992), published further results concerning treating potato starch at ambient temperature (20-21°C). It was observed that new

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types of limit dextrans can be produced by an appropriate choice of alcohol and concentration of acid.

Thus, despite prior efforts, there still exists the need for a carbohydrate source for nutritional

5 supplements that is capable of satisfying the demanding physiological-related criteria described herein for such an application, as well as possessing the appropriate physical characteristics needed for commercial utility, while providing desired processing characteristics.

10 It is accordingly a principal object of the present invention to provide a starch material having the physiological responses satisfactory for use in nutritional food formulations.

15 A further and more specific object of this invention provides modified starches having desired glycemic, endocrine, and metabolic responses in humans under a variety of physiological conditions.

20 Yet another object lies in the provision of modified starches having physical characteristics satisfactory to allow use in commercial nutritional food applications.

Another object provides nutritional products containing such starches.

25 Yet another object is to provide a method for making such starches. A related and more specific object provides modified starches having acceptable water solubility characteristics.

A still further and more specific object is to provide a method for making such starches having altered, and desired, processing characteristics.

30 Other objects and advantages will become apparent as the following description proceeds.

SUMMARY OF THE INVENTION

In accordance with the present invention, it has
35 been found that starches which are appropriately acid-treated yield modified starches that possess extremely desirable physiological characteristics when employed as

an energy source for nutritional products. More particularly, such modified starches cause only a relatively slight degree of osmolarity and a low insulin response while achieving desirable blood glucose 5 responses. A particularly preferred energy source, due to its oxidation and digestibility characteristics, can be achieved by utilizing modified corn starch, more preferably modified amylo maize-7 corn starch.

Still further, it has been discovered that starches 10 that are appropriately acid-treated provide modified starches having altered, and desired, processing characteristics, such as gelling and/or paste profile characteristics.

15

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph of the molecular weight of modified corn starch versus concentration of acid and illustrating the effect on the resulting molecular weight as the acid concentration is varied using different 20 levels of the starting, unmodified corn starch;

FIG. 2 is a graph of the molecular weight of the modified corn starch versus acid concentration when using certain alcohols as the reaction medium;

FIG. 3 is a graph similar to FIGURE 1, except 25 illustrating the effect of the starting, unmodified starch level on molecular weight when potato starch is used;

FIG. 4 is a graph similar to FIG. 2, except showing the effects when potato starch is utilized; and

30 FIG. 5 is a graph similar to FIG. 4, except employing other alcohols as the reaction medium.

DETAILED DESCRIPTION OF THE INVENTION

Modified starches suitable for use in the present 35 invention should have a number average molecular weight of at least about 10,000. Preferably, the molecular weight will be in the range of about 20,000 to 40,000,

even more preferably about 30,000 or so. Modified starches having molecular weights in excess of about 90,000 or more will not typically provide the characteristics desired for a nutritional product. For 5 the purposes herein, number average molecular weight may be determined by preparing a solution containing 10.0 mg/ml of starch, and determining reducing values by the Somogyi-Nelson, alkaline-copper procedure or the copper bicinchoninate microprocedure, using maltose as the 10 standard. The number average molecular weight can be obtained by the following:

$$\overline{MW}_n = \frac{\text{total carbohydrate} (\mu\text{g})}{\text{reducing value} (\mu\text{g of maltose})} \times 342$$

where the total carbohydrate is obtained by a phenol- 15 sulfuric acid analysis, Dubois et al., "Colorimetric Method for Determination of Sugars and Related Substances," Anal. Chem., 28, 350-353 (1956); Fox and Robyt, "Miniaturization of three carbohydrate analyses using a microsample plate reader," Anal. Biochem., 195, 20 93-96 (1991), and where the reducing value is determined using the Somogyi-Nelson alkaline-copper procedure, Robyt and Whelan, "Starch and Its Derivatives," pp. 431-432, 4th ed. (Chapman and Hall, London, 1968), or by the copper-bicinchoninate microprocedure, Fox and Robyt, Anal. Biochem., 195, 93-96 (1991). Alternatively, the number 25 average molecular weight may be determined from the average degree of polymerization (d.p.) using the following relationships:

$$\overline{d.p.} = \frac{\text{total carbohydrate} (\mu\text{g})}{\text{reducing value} (\mu\text{g of maltose})} \times 2$$

$$\overline{MW}_n = (\overline{d.p.}) \times 162$$

Both methods provide the same number average molecular weight values, as has been determined from experience 35 relating to the modified starches described herein.

In addition, when used as a liquid nutritional supplement, suitable modified starches should have sufficient solubility in water so that the volume of liquid that one would have to consume to achieve the 5 desired metabolic benefit would not be unduly excessive. While some applications might find as little as 5 mg/ml or even 2 mg/ml suitable, it will generally be desirable to have a water solubility of at least 10 mg/ml, which will be satisfactory for many applications.

10 In general, when the modified starches of this invention are used as a liquid nutritional supplement, the higher the water solubility is the better. Ideally, the water solubility of these modified starches would be 30 mg/ml or more. The relative water solubility of the 15 resulting modified starch will be determined by the treating process used, as will be discussed hereinafter.

An appropriate method for determining the level of water solubility is the phenol/sulfuric acid method. Dubois et al., Anal. Chem., 28, 350-353 (1956). In 20 general, this method comprises suspending 500 mg of the modified starch in 2 ml of water, autoclaving the resulting suspension at 121°C for 30 minutes, and then allowing the autoclaved suspension to cool to room temperature. The remaining undissolved starch is then 25 removed by centrifugation, and a total carbohydrate analysis, using the phenol/sulfuric acid method, is carried out on the supernatant.

Suitable modified starches can be obtained by 30 treating the starch of choice with hydrochloric acid in a lower alcohol and coordinating the treating parameters so as to yield the desired molecular weight. Achieving modified starches with molecular weights useful for the present invention should likewise result in a satisfactory degree of water solubility. However, the 35 water solubility can be determined as previously described so as to insure that the solubility is in fact appropriate.

- In general, the parameters which should be coordinated involve the type and relative amount of the starch to be treated, the selection of the lower alcohol, the treating temperature, the time, and the amount of the hydrochloric acid used. As to the type of starch, any starch source may be used which, upon treating, provides a modified starch of suitable molecular weight that also imparts the desired metabolic benefits desired for the particular application, as will be discussed hereinafter.
- Illustrative examples of starch sources include corn, potato, oat, rice, and wheat. Preferably, corn starch is used, and, even more preferably, amylo maize-7. It is, thus, preferred to use starch sources that possess a relatively high amylose content.
- Regarding the relative amount of the starch in relation to the volume of the lower alcohol employed, the amount used can be varied as is desired for the treating process efficiency. By way of example, it will be suitable to use starting starch levels of from about 0.25 to 1.0 kg/liter of the lower alcohol.
- Suitable lower alcohols to serve as the treating medium include, for example, methanol, ethanol, propanol (e.g., 2-propanol), and butanol (e.g., 1-butanol). As may be appreciated, it may be more desirable to utilize ethanol for making the modified starches for the nutritional products of this invention. However, any of the other lower alcohols can be used; but, when used, a wash should be carried out to remove the acid and alcohol. As one example, a 50% v/v water-ethanol wash can be used.
- The treating temperature may be varied from ambient (i.e., 21°-22°C.) to about 60°C. or so. For ease of preparation and to avoid the expense of heating, ambient conditions are desirable. The treating time can be varied as desired to allow the treatment to reach completion. A treating time of three days has been found to be satisfactory.

The level of hydrochloric acid employed can be varied from about 10 up to 100 ml/liter of the lower alcohol. Typically, the molecular weight of the modified starch will be reduced with increasing amounts of 5 hydrochloric acid.

The coordination of the treating parameters will be further discussed in the Examples. As will be seen, modified starches having the desired molecular weight and solubility characteristics can be readily provided.

10 Use of the thus-modified starches of the present invention as nutritional supplements will exert only a slight degree of osmolarity. More particularly, an osmolarity (measured using freeze-point osmolarity) of no more than about 500 mOsm/l can be provided. Preferably, 15 the modified starches utilized should have an osmolarity of no more than about 200 mOsm/l, even more preferably, no more than 100 mOsm/l.

Thus, in comparison to the carbohydrates previously used as nutritional supplements, the modified starches of 20 this invention impart highly desirable osmolarity characteristics. Indeed, the slight osmolarity that is exerted should allow the modified starches to significantly minimize, and perhaps even eliminate, undesirable gastrointestinal effects such as diarrhea and 25 distention.

Another aspect of this invention is the superior insulin response characteristics that are achieved by using the modified starches of this invention in comparison to the insulin responses that occur with 30 previously used carbohydrates. In general, consistent with having satisfactory digestibility to provide the desired caloric or energy value, the lower the insulin response that results, the better.

In accordance with this invention, in comparison to 35 glucose, the modified starches result in insulin responses no more than about 50% of the insulin responses resulting from using glucose. Preferably, the modified

starches of the present invention result in insulin responses of no more than about 10%, or even less. The insulin response comprises the rise in blood insulin concentration from the pre-feeding concentration to the 5 peak concentration (usually occurring within one hour after feeding), and the rise associated with the feeding of the modified starch is expressed as a percentage of the rise observed when an equivalent mass of glucose is fed to the same person under the same conditions.

10 The modified starches useful in this invention likewise exhibit highly desirable blood glucose response characteristics in comparison to those resulting from using glucose. More specifically, the blood glucose response (peak) resulting when the modified starches of 15 this invention are utilized is no more than 50%, preferably no more than 12% and, even more preferably, no more than 0%, while the blood glucose response (area) is no more than 75%, preferably no more than 30%, and, even more preferably, no more than 10% of the blood glucose 20 response resulting when dextrose or glucose is used. The blood glucose response (peak) is defined as the rise in blood glucose concentration from the pre-feeding concentration to the peak concentration (usually occurring within one hour after feeding), expressed as a 25 percentage of the rise observed when an equivalent mass of glucose is fed. The blood glucose response (area) consists of the total area under the curve of blood glucose concentration (millimolar) over a three-hour period after ingestion of 75 gms suspended in 350 ml 30 water, the subjects being tested following a 12 hour fast. The total area of the modified starch feeding is expressed as a percentage of the total area obtained after feeding an equivalent mass of glucose. The foregoing assumes that the blood glucose concentration 35 does not fall below the pre-ingestion level at any time during the three-hour test. More particularly, in a small percentage of the population (perhaps 10 to 20%),

the high insulin produced with glucose will cause the glucose level to not only fall to the pre-ingestion level, but to below that level. Consequently, such a precipitous drop can decrease the area under the blood
5 glucose curve, thereby making any comparison inaccurate.

Suitable protocols for determining the physiological characteristics described herein are set forth below:

Glycemic response protocol

10 Ten healthy college-aged (25 ± 1 yr.) subjects participate (6 males, 4 females). The subjects are individually screened for participation and eliminated from the study if diabetes or a history of elevated fasting blood glucose concentration is found. In
15 addition, subjects are eliminated from the study if they were taking any medications known to affect blood glucose homeostasis and/or insulin responses to meals.

The subjects are required to record all food and beverages consumed for two days prior to their first
20 trial and then to duplicate this diet for two days prior to each subsequent trial. Before participating in the first trial, the subject's diet is analyzed using Nutritionist III™ for total calories, grams of carbohydrate, grams of fat, and grams of protein. The
25 results of the two-day diet analysis are averaged on a per day basis. If a subject's diet analysis revealed that his or her dietary intake of carbohydrates was less than 150 g/day, his/her trial is postponed and the subject is instructed to eat more carbohydrates for
30 several (2-3 days) days before the rescheduled trial.

In addition, the subjects are required to fast overnight (no food, calorie-containing beverages, or caffeine) and to abstain from any exercise or alcohol for 24 hours prior to each experimental trial. Finally,
35 water intake is prohibited during the one hour before each experimental trial in order to assist with the standardization of fluid volume in the stomach.

When the subject reports to the laboratory for each experimental trial, a flexible Teflon catheter is inserted into a prominent forearm vein. To prevent blood from clotting in the catheter, 2 ml of sterile saline 5 (0.9% sodium chloride for injection) is infused into the catheter which is capped with a sterile 3-way stopcock. Blood samples are obtained from the catheter immediately before ingestion of the experimental solution, 30 minutes after ingestion is complete, 60 minutes after ingestion, 10 90 minutes after ingestion, 120 minutes after ingestion, and 180 minutes after ingestion. Prior to each blood sample, 4 ml of blood is withdrawn from the catheter and discarded to remove the saline left in the stopcock and catheter. The blood samples are taken with heparinized 15 syringes and transferred into labelled test tubes. The samples are then immediately centrifuged at 2000 xg for minutes to separate the plasma from the red blood cells. The plasma is then removed, transferred to a separate tube and frozen at -20°C where the sample is stored for 20 later analysis of glucose and insulin concentration.

The experimental solutions containing the carbohydrate being tested are administered in a randomized, double-blind manner. Each solution is prepared using 75 grams of the carbohydrate mixed in 300 25 ml water at room temperature. The solution is sipped through an opaque straw from a covered opaque container. The solution is swirled immediately before drinking to ensure thorough mixing of contents and then sipped continuously over a two-minute period. Once the 30 container is emptied, 60 ml of water is added to the container and swirled to mix with any remaining solids and ingested by the subject. All experimental trials are begun between 7:00 and 9:00 AM. Start time is assessed as the time when the subject began ingesting the 35 experimental solution. During the first trial, this time is noted and duplicated for that subject on each subsequent trial.

- Once all experimental trials for all subjects are complete, plasma samples are analyzed. Plasma glucose concentration is measured in duplicate on each sample using a YSI 2300 Stat Glucose/Lactate Analyzer (Yellow Springs Instruments, Yellow Springs, OH). These duplicate measurements are averaged for further analysis. Plasma insulin concentration is measured using double-antibody ^{125}I iodine radioimmunoassay kits for insulin supplied by ICN Biomedicals, Inc. (Costa Mesa, CA).
- 10 Radioactivity is counted on a Packard Cobra II Auto-Gamma Counter. Each plasma insulin determination is made in triplicate, and the average of the three determinations is used in subsequent analyses.

For each experimental trial on each subject, the area under the plasma glucose curve is measured. The geometric area above resting, pre-ingestion, plasma glucose concentration, is measured by entering the glucose concentrations that are obtained at the designated times after ingestion into a computer program that calculates area under curves using a trapezoidal model (SigmaStat 4.1, Jandel Scientific, San Rafael, CA). Because this procedure defines only the area above pre-ingestion plasma glucose concentration, it will be referred to as hyperglycemic area. To calculate the glycemic index, the hyperglycemic area occurring after ingestion of the experimental solution is expressed as a percentage of the hyperglycemic area occurring after ingestion of the glucose solution.

To characterize the plasma insulin responses of each subject to each of the experimental solutions, the amount of increase in plasma insulin concentration from pre-ingestion to peak insulin concentration irrespective of time is calculated for each subject under each treatment. This calculated increase is expressed as a percentage of the calculated increase that occurred when the glucose solution is ingested.

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The plasma glucose concentrations that are obtained after ingestion of the experimental solutions are compared for significant differences between treatments and within treatments using a two-way analysis of variance for repeated measures. If a significant F-ratio is obtained in the analysis of variance, a Newman-Kuel multiple range test is used to determine between which treatments and/or between which times within treatments significant differences occurred. The insulin 5 concentrations are analyzed in the same way.

Once the calculated data are obtained (hyperglycemic areas, glycemic index, and insulin responses) for each subject under each treatment, the data is examined for significant differences between treatments using 10 conventional statistical methods. Hyperglycemic area is compared between glucose and the carbohydrate, or carbohydrates, being tested using a one-way analysis of variance for repeated measures. If a significant F-ratio was obtained in the analysis of variance, a Newman-Kuel 15 multiple range test is used to determine between which treatments significant differences occurred. Glycemic index is tested for significant differences between the test carbohydrates using a paired t-test. Insulin responses are compared for significant differences 20 between the test carbohydrates using a one-way analysis of variance for repeated measures. If a significant F-ratio is obtained in the analysis of variance, a Newman-Kuel multiple range test is used to determine between which treatments significant differences occurred. For 25 all statistical tests, the level of significance that is used to determine if a significant difference occurred is p < 0.05.

Metabolic Protocol - Oxidation During Exercise

35 Ten healthy (28 ± 2 yr) male subjects participate. The subjects are individually screened for participation and eliminated from the study if diabetes or a history of

elevated fasting blood glucose concentration is found. In addition, subjects are eliminated from the study if they are taking any medications that are known to affect blood glucose homeostasis and/or insulin responses to 5 meals.

The subjects are required to record all food and beverages consumed for three days prior to their first trial and then to duplicate this diet for three days prior to each subsequent trial. Before participating in 10 the first trial, the subject's diet is analyzed using Nutritionist III™ for total calories, grams of carbohydrate, grams of fat, and grams of protein. The results of the three-day diet analysis are averaged on a per day basis. If a subject's diet analysis revealed 15 that his dietary intake of carbohydrates was less than 150 g/day, his trial is postponed and the subject is instructed to eat more carbohydrates for several days before the rescheduled trial.

In addition, subjects are required to fast overnight 20 (no food, calorie-containing beverages, or caffeine) and to abstain from any exercise or alcohol for 24 hours prior to each experimental trial. Finally, water intake is prohibited during the one hour before each experimental trial in order to assist with the 25 standardization of fluid volume in the stomach.

At least one week before starting the experimental trials, the subjects are tested for maximum oxygen uptake (VO₂max) on a cycle ergometer. VO₂max is determined using a graded exercise test starting with 4 minutes of 30 pedaling at 60 rpm and no workload. Following this initial warm-up period, the subjects maintain the 60 rpm while the workload is increased by 25 watts every three minutes. The test is continued in this manner until the subject can no longer keep his pedal rate at the 35 prescribed 60 rpm. Oxygen uptake is measured continuously during the exercise test using indirect calorimetry. Inspired air volumes are measured using a

Parkinson-Cowan CD-4 dry gas meter, and expired air is collected in a three liter mixing chamber. Expired air samples are collected continuously from the mixing chamber and analyzed for oxygen and carbon dioxide 5 concentrations (Applied Electrochemistry SA-2 Oxygen Analyzer and Beckman LB-2 CO₂ Analyzer, respectively). These analyzers are calibrated immediately before each exercise test using known gas standards. The electrical output from the O₂ analyzer, CO₂ analyzer, and the gas 10 meter are routed into an A-D converter connected to an Apple IIe computer. These signals are processed, and oxygen uptake is calculated for each 30 second period in exercise using commercial VO₂ software (REP-200B, Rayfield Equipment, Inc.). Maximum oxygen uptake is taken as the 15 highest VO₂ achieved during the exercise test. Oxygen uptake at each workload is calculated as the average VO₂ during the last minute of each workload. The workload corresponding to 70% VO_{2max} for each subject is estimated using a linear regression between VO₂ and workload. This 20 workload is then used during the subsequent experimental trials.

When the subject reported to the laboratory for each experimental trial, a flexible Teflon catheter is inserted into a prominent forearm vein. To prevent blood 25 from clotting in the catheter, 2 ml of sterile saline (0.9% sodium chloride for injection) is infused into the catheter which is capped with a sterile 3-way stopcock. Blood samples are obtained from the catheter immediately before ingestion of the experimental solution (pre-ingestion), 30 minutes after ingestion was complete (pre-exercise), 30 minutes into exercise, and 60 minutes into exercise. Prior to each blood samples, 4 ml of blood is withdrawn from the catheter and discarded to remove the saline left in the stopcock and catheter. The blood 30 samples are taken with heparinized syringes and transferred into labelled test tubes. The samples are then immediately centrifuged at 2000 xg for 10 minutes to 35

separate the plasma from the red blood cells. The plasma is then removed, transferred to a separate tube and frozen at -20°C where the sample is stored for later analysis of glucose concentration, insulin concentration, 5 and lactate concentration.

The experimental solutions containing the test carbohydrates are administered in a randomized, double-blind manner. Each solution is prepared using 75 grams of the carbohydrate mixed in 300 ml artificially 10 sweetened lemon drink (Wyler's) at room temperature. The placebo consists of only the lemon flavored drink. The solution is sipped through an opaque straw from a covered opaque container. The solution is swirled immediately before drinking to ensure thorough mixing of contents and 15 then sipped continuously over a two-minute period. Once the container is emptied, 60 ml of the lemon drink is added to the container and swirled to mix with any remaining solids and ingested by the subject. All experimental trials are begun between 7:00 and 9:00 AM. 20 Start time is assessed as the time when the subject began ingesting the experimental solution. During the first trial, this time is noted and duplicated for that subject on each subsequent trial.

Muscle biopsy samples are obtained immediately 25 before and after each experimental exercise bout from the vastus lateralis muscle using the needle biopsy procedure described by Bergstrom [Bergstrom, "Muscle electrolytes in man.," Scandinavian Journal of Clinical Investigation, 14, 1-110 (1962)]. After each biopsy is taken, the sample is 30 dipped in liquid nitrogen to quick-freeze the sample and is stored in liquid nitrogen until later analysis of glycogen concentration. Once all of the experimental trials are completed, each biopsy sample is dissected at -20°C into three separate 5-10 mg pieces, weighed while 35 frozen on a microbalance, and stored. Glycogen concentration of each of the three pieces is performed using the enzymatic method described by Passonneau

(Passonneau et al., "A comparison of three methods of glycogen measurement in tissues," Anal. Biochem., 60, 405-412 (1974)). The glycogen concentration measurements obtained on the three pieces of the same muscle sample 5 are average, and this average concentration is used in further analysis. Glycogen use during exercise is measured as the difference in glycogen concentration between the pre-exercise and post-exercise muscle samples.

10 Thirty minutes after the subject ingests the experimental solution and immediately after the pre-exercise biopsy is taken, a 3-5 minute unloaded warm-up period on the cycle ergometer is allowed. At the end of this warm-up period, the workload is immediately set at 15 the predetermined workload corresponding to 70% of that subject's VO₂max. This exercise is continued for 60 minutes. During the exercise test, VO₂ is measured as described above during minutes 0-5, 25-30, and 55-60. The average VO₂ (L/min) and respiratory quotient (RQ = 20 VCO₂/VO₂) obtained during the last two minutes of each of these periods are used in calculating carbohydrate oxidation rates. Blood samples are also obtained during these time periods. Within five minutes after the conclusion of the 60-minutes exercise, the post-exercise 25 muscle biopsy is performed.

Once all experimental trials for all subjects are completed, plasma samples are analyzed. Plasma glucose and lactate concentrations are measured in duplicate on each sample using a YSI 2300 State Glucose/Lactate 30 Analyzer. The duplicate measurements are averaged for further analysis.

Carbohydrate oxidation rates (g/min) are calculated for each trial of each subject using the VO₂ and RQ data obtained during the 60-minute exercise bout. To 35 calculate the total carbohydrate oxidation, the VO₂ and RQ results (expressed on a per minute basis) are obtained at three time periods (0-5 min, 25-30 min, and 55-60 min).

During each of these time periods, average VO_2 and average RQ are calculated. From these data, carbohydrate oxidation rate (grams per liter of oxygen consumed per minute) is estimated using the thermal equivalents for 5 oxygen as described by Zuntz (Zuntz, Pflugers Archives of Physiology, 83, 557 (1901)). The resultant average carbohydrate oxidation obtained in the 0-5 minute period is multiplied by 5 to yield total carbohydrate oxidized in the first 5 minutes of exercise (g/5 min). The 10 averaged rate of carbohydrate oxidation obtained in the 25-30 minute period is multiplied by 25 to yield total carbohydrate oxidized from 6 minutes until 30 minutes (g/25 min). The averaged rate of carbohydrate oxidation obtained in the 55-60 minute period is multiplied by 30 15 to yield total carbohydrate oxidized in the second 30 minutes of the exercise (g/30 min). The three total carbohydrate estimates are then added to yield the carbohydrate oxidized during the entire 60-minute exercise bout (g/hr).

20 Total carbohydrate oxidation (g/hr) is then compared among the treatments by first calculating the difference in carbohydrate oxidation between the placebo trial and the glucose trial. This provides a measurement of the amount of increase or change in carbohydrate oxidation 25 that is produced by a glucose feeding. The differences in carbohydrate oxidation between placebo trial versus test carbohydrate(s) are also computed. The change in carbohydrate oxidation for each test carbohydrate is then expressed as a percentage of the change in carbohydrate 30 oxidation that is produced by the glucose feeding.

For each experimental trial on each subject, the area under the plasma glucose curve is measured. The geometric area above resting (pre-ingestion) plasma glucose concentration is measured by entering the glucose 35 concentrations that are obtained at the designated times after ingestion into a computer program that calculates area under curves using a trapezoidal model (SigmaStat

4.1). Because this procedure defines only the area above pre-ingestion plasma glucose concentration, it will be referred to as hyperglycemic area.

The plasma glucose and lactate concentrations that
5 are obtained after ingestion of the experimental
solutions are compared for significant differences
between treatments and within treatments using a two-way
analysis of variance for repeated measures. If a
significant F-ratio is obtained in the analysis of
10 variance, a Newman-Kuel multiple range test is used to
determine between which treatments and/or between which
times within treatments significant differences occurred.

Hyperglycemic areas, muscle glycogen use, and total
carbohydrate oxidation (g/hr) for each subject under each
15 treatment are examined for significant differences
between treatments using conventional statistical
methods. Hyperglycemic area, muscle glycogen use, and
total carbohydrate oxidation (g/hr) are compared between
glucose, the test carbohydrate(s), and placebo using a
20 one-way analysis of variance for repeated measures. If a
significant F-ratio is obtained in the analysis of
variance, a Newman-Kuel multiple range test is used to
determine between which treatments significant
differences occurred. For all statistical tests, the
25 level of significance that was used to determine if a
significant difference occurred is $p < 0.05$.

As has been previously alluded to herein, useful
modified starches should have adequate digestibility so
that caloric or energy values are provided when used. In
30 accordance with this invention, the modified starches
that are useful exhibit a digestibility of at least about
50%, preferably 90%, and, even more preferably, 100%.
This characteristic is determined as the percentage of
the modified starch material fed that is not excreted as
35 carbohydrate in feces for a three-day period. A suitable
protocol to determine digestibility is set forth in
Example 7 hereinafter. It has been found that modified

corn starch, particularly amylo maize-7, tends to exhibit more desirable digestibility characteristics as compared to modified potato starches.

In accordance with the preferred embodiment of this invention, the modified starches utilized preferably have desirable metabolic characteristics. One method of measuring one important metabolic fate is via determining the oxidation characteristics. More particularly, the preferred modified starches of the present invention possess oxidation characteristics of at least 50%, preferably at least 100%, and, even more preferably, at least about 200%, the percentages being measured as hereinafter set forth. Such carbohydrate oxidation, indicated as grams per hour, is that which results during one-hour of cycle exercise at an intensity level of 70% of the maximum oxygen uptake. This is measured using indirect calorimetry. The difference in carbohydrate oxidation between a placebo and the modified starch ingestion is expressed as a percentage of the difference in the carbohydrate oxidation between the placebo and a glucose injection. The dose used is as was described in conjunction with the blood glucose response, i.e., 75 grams of the starch suspended in 350 ml of water. This dosage is ingested 30 minutes before the exercise.

Such oxidation characteristics thus are one method of determining that the modified starches behave the same way as does glucose. Further, the levels of digestibility and oxidation provide one method of assessing the relative caloric or energy value efficiency of an energy supplement. The preferred modified starches of the present invention achieve appropriate oxidation levels as well as digestibility. However, modified starches having satisfactory digestibility are suitable regardless of oxidation characteristics, as such materials can still achieve mutable caloric values through metabolic pathways other than oxidation.

In accordance with yet another aspect of the present invention, it has been discovered that appropriate acid-treating as described herein, yields modified starches having altered, and desired, processing characteristics.

- 5 More particularly, the physicochemical properties of starches are determined, in part, by the relative proportions of amylose and amylopectin contained in the starch granule.

Thus, starches with a relatively high proportion of
10 amylose, such as amylo maize-7 (70% amylose), tend to exhibit a high gelatinization temperature and set to form a firm gel upon cooling. In contrast, waxy maize starch is known to set easily and sets to a clear gel when cool. On the other hand, the pasting characteristics for waxy
15 maize starch tend to have undesirably high peak and hot paste viscosity characteristics.

Selection of the appropriate starch and acid-treating using the method described herein allow a modified starch to be provided with processing
20 characteristics tailored for the processing requirements of a particular application. To this end, the gelling characteristics may be altered in the modified starches so as to reduce by 1 to 10° C relative to unmodified starch the temperature required to initiate gel formation
25 (defined as the onset of peak during differential scanning colorimetry, DSC, as will be described in detail hereinafter, and to reduce the energy required to produce a gel by 1 to 50% (the energy required being defined as enthalpy during DSC as discussed hereinafter)).

30 Further, in those applications where retrograded starch is desired, The temperature required to initiate gel formation of the modified, retrograded starch can be reduced by 1 to 30°C. (defined as peak onset during DSC analysis of the retrograded starch).

35 Also, the modified starches possess altered pasting profile properties. Thus, pursuant to this aspect of the present invention, appropriate selection of the modified

starches as determined by a Rapid Visco Analyzer (RVA) can provide substantial reduction in pasting viscosities and the like relative to unmodified starches. To this end, such modified starches possess peak and hot paste viscosity reduced by 50 to 100%, as are the: (1) breakdown of starch paste upon continued heating after obtaining peak viscosity (2) viscosity of starch paste upon cooling after heating, and (3) setback of starch pastes.

Modified starches having such reduced paste viscosity and other pasting properties relative to unmodified starches should provide advantageous properties for various applications. Thus, such reduced pasting properties should allow greater facility during processing and use.

The characteristics achieved by the modified starches of the present invention allow usage in any liquid nutritional product. An illustrative list of examples of the type of formulas include infant formulas, liquid enteric formulations for patients with comprised abilities to ingest food (viz., patients with nasogastric tubes and/or intestinal tubes), liquid supplements for use by, before, during, or after exercise, oral dietary aids to prevent hypoglycemia in certain types of genetic-based glycogen storage diseases, dietary aids for non-insulin dependent and insulin-dependent diabetic patients, oral dietary aids for carbohydrate-sensitive individuals (i.e., sensitive to existing carbohydrate formulations), and use in snack foods such as candies, cookies, cakes, pies, either alone or in combination with simple sugars and/or artificial sweeteners. Common to these nutritional products is an edible carrier, often a liquid such as water. In addition, such nutritional products can include any of the other typically used ingredients such as, for example, flavoring agents, emulsifiers, phosphates, salts, coloring agents, artificial colors, fats,

proteins, sugars, artificial sweeteners, amino acids, preservatives, vitamins, minerals, baking soda, baking powder, other carbohydrates or starches, and pharmaceutical agents.

5 Indeed, the modified starches of the present invention may also be used in products which are in tablet or powder form. For example, oral dietary aids and certain snack foods may be desirably used in other than a liquid form. In this connection, the procedure
10 for making the modified starches provides such starches in a dry powder form.

The relative amount of the modified starch used in the nutritional product is a nutritionally effective amount, i.e., that amount which will provide the caloric
15 or energy value desired for the particular product. Typically, liquid products will use at least about 2 mg/ml, up to about 30 mg/ml, or more, while solid products will use one percent by weight or more, based on the total weight of the product.
20

The following Examples are illustrative, but not in limitation of, the present invention. As used in the following Examples, the following terms and tests may be described as follows:

25 Randomized, double blind fashion: A standard experimental design in which a group of subjects is assigned to be given several treatments in a randomized order and neither the subjects nor the investigator knows who is receiving which treatment in what order. Instead,
30 treatments are coded (e.g., A, B, C, D etc.) and the key to the code is not disclosed until the experiment is completed.

35 Blood glucose concentration: Standard quantification of the glucose level in blood. Units used are mM (millimoles per liter).

Hyperglycemic area: Ingestion of a carbohydrate typically increases the blood glucose concentration above

baseline values (baseline is usually 4-5 mM) for 2-3 hours. The difference between blood glucose concentration and baseline concentration is graphed as a function of time and the hyperglycemic area is the 5 geometric area under this curve. Units are mM x min.

Glycemic index: This represents the size of the blood glucose concentration response after ingestion of the experimental substance. Size is measured as the hyperglycemic area (see above) and is expressed as a 10 percentage of the hyperglycemic area resulting from a dextrose/glucose feeding in the same subject under identical conditions.

Serum insulin concentrations: Concentration of insulin in serum obtained after centrifuging off the red 15 blood cells. Units are μ IU/mL (micro-International Units per mL).

VO₂max: Maximum ability to consume oxygen. This is measured herein as peak oxygen uptake during incremental continuous cycle ergometer test to volitional exhaustion. 20 More specifically, the subject pedals on a stationary bike at a prescribed rate (60 rpm herein), beginning with no pedal resistance. Then, every three minutes the pedal resistance is increased until the subject can no longer maintain the prescribed pedal rate. During the test, 25 oxygen uptake is measured continuously using indirectly calorimetry (standard method); and the highest oxygen uptake is taken as the VO₂max.

Muscle glycogen use: The positive difference in muscle glycogen concentration between pre-exercise and 30 immediately post-exercise.

Respiratory quotient: As used herein, this is the ratio of carbon dioxide produced per minute to oxygen consumed per minute (VCO_2/VO_2). This is a standard measurement obtained from indirect calorimetry and allows 35 the proportion and quantity of carbohydrate being oxidized by the body during exercise to be determined.

X ± SEM: Group mean (average) plus or minus one standard error of the mean (SEM = standard derivation divided by square root of n minus one, where n is the number of subjects).

- 5 ANOVA: Analysis of variance.

DSC Analysis

A DSC 7 analyzer equipped with a thermal-analysis data station (Perkin-Elmer Corp., Norwalk, CT) was used.

10 Analysis of starch gelatinization was conducted as described by White et al., "Intra- and Interpopulation Variability of Thermal Properties in Maize Starch," Cereal Chemistry, 67:70-73 (1990). Approximately 4.0 mg (dry weight) of starch were weighed into aluminum sample

15 pans, and 8 mg of distilled water were added. Samples were heated from 30 to 102°C at a rate of 10°C/min. DSC parameters included enthalphy (ΔH), gelatinization onset temperature T_o , T_p and ΔH were given directly by the DSC software. Range was calculated by ($T_p - T_o$) according to

20 Krueger et al., "A Differential Scanning Calorimetry Study on the Effect of Annealing on Gelatinization Behavior of Corn Starch," J. Food Sci., 52:715-718 (1987). Samples were stored for seven days at 4°C and rerun to determine starch gel retrogradation (%R) as

25 described by White et al., "Freeze Thaw Stability and Refrigerated Storage Retrogradation of Starches," Starch/Staerke, 41:176-180 (1989). This methodology does not account for possible contribution of amylose-lipid complexes that may affect gelling characteristics of high

30 amylose starches (Amy-5 and Amy-7 in the present study).

RVA Pasting Profiles.

These were constructed using a Rapid Visco Analyzer model 4 (RVA-4, Newport Scientific Pty., Ltd.,

35 Warriewood, NSW, Australia) and at STD2 temperature profile equipped with Thermociline for Windows software version 1.2. Between three and five RVA profiles were

obtained for each starch type. For each determination, a 9.3% starch suspension in distilled water was used. Final slurry weights were 30 g. The STD2 temperature profile involved heating the starch-water mixture for 1 5 min at 30° C to equilibrate the sample, followed by increasing the temperature to 95°C in 10 min. The temperature was held at 95°C for 5.7 min, decreased to 50°C in 7.3. min, and held at 50° for 2 min, for total of 26 min per run.

10

EXAMPLE 1

This Example shows the effect on the molecular weight of varying the concentration of the starch being modified. A series of corn starch samples (amylo maize-7) 15 were suspended in one liter of ethanol for three days at 20°-22°C. while various concentrations of hydrochloric acid were used and the level of the starting or unmodified starch was varied from 0.25 kg to 0.5 kg and to 1 kg per liter of ethanol. The relative amounts of 20 starch and the levels of hydrochloric acid are set forth in Table 1 together with the resulting number average molecular weight of the modified starches:

Table 1

HCl		Amount of High Amylose Corn Starch		
(%w/v)	(mL/L)	1 Kg	0.5 Kg	0.25 Kg
0.36	10	54108 ± 2268	50706 ± 3402	39204 ± 1782
0.71	20	52650 ± 3564	45360 ± 1620	36288 ± 2430
1.05	30	50544 ± 2430	40662 ± 1944	35487 ± 810
1.39	40	49410 ± 2268	34830 ± 2754	30456 ± 1085
1.71	50	46008 ± 1944	34344 ± 2106	29160 ± 1620
2.04	60	41148 ± 1620	34020 ± 2430	24786 ± 988
3.27	100	37260 ± 1166	29970 ± 2268	23652 ± 1490

25

The results are set forth in graph form in FIGURE 1.

As can be seen in FIGURE 1, all of the resulting modified high amylose starch materials exhibited number average molecular weights within the range useful in the present invention. Further, at a given level of the starting starch material, the molecular weight decreases with the use of increasing levels of the hydrochloric acid. By appropriate selection of the level of starting starch material, the desired molecular weight and the relative amount of the hydrochloric acid used, modified high amylose starch materials having the desired molecular weight can readily be obtained.

EXAMPLE 2

This Example illustrates the effect of using differing lower alcohols as the treating or reaction medium.

A series of high amylose corn starch samples (amylo maize-7) were acid treated at 20°-22°C. for three days at a level of 0.25 kg/liter of the lower alcohol employed. Four series of the high amylose corn starch samples were treated using methanol, ethanol, 2-propanol, and 1-butanol. The number average molecular weights were determined and the parameters and resulting molecular weights are shown in Table 2:

25

Table 2

HCL Concentration		Alcohol			
(%w/v)	(mL/L)	MeOH	EtOH	2-PrOH	1-BuOH
0.36	10	48924 ± 2754	39204 ± 1782	20412 ± 1118	16200 ± 405
0.71	20	46980 ± 2916	36288 ± 2430	18306 ± 1037	14126 ± 551
1.39	40	45846 ± 1944	30456 ± 1085	17172 ± 1377	12442 ± 389
2.67	80	45846 ± 2430	24138 ± 1037	17010 ± 486	10481 ± 275
5.0	160	35964 ± 2268	21060 ± 1069	12814 ± 373	9007 ± 324

The results are graphed in FIG. 2 which illustrates the varying molecular weight with the various levels of hydrochloric acid for the alcohols utilized.

As can be seen, the resulting number average
5 molecular weight of the thus-modified high amylose corn starch is the highest when methanol is used and then decreases progressively for ethanol, 2-propanol and 1-butanol, respectively.

10

EXAMPLE 3

This Example is similar to Example 1 with the exception of showing the effect of the starch concentration on the resulting molecular weight of the acid-modified starch when potato starch is used.

15 Three series of potato starch samples, each at starting levels of 0.25 kg, 0.5 kg, and 1 kg, were subjected to various amounts of hydrochloric acid while suspended in one liter of ethanol for three days at a temperature of 20°C. The resulting molecular weights
20 were determined, and the results are set forth in Table 3:

Table 3

HCl		Amount of Starch/Liter of Ethanol		
(%w/v)	(mL/L)	1 Kg	0.5 Kg	0.25 Kg
0.36	10	314,000±2700	187,000±1500	85,000±1000
0.71	20	182,000±2000	100,500±1200	40,000± 700
1.05	30	130,000±1500	72,000±1200	37,000± 500
1.39	40	111,000±1200	67,000±1200	35,000± 500
1.71	50	100,000±1200	66,000±1200	34,000± 450
2.04	60	67,000±1000	60,000±1000	30,000± 400

25 The graph of the molecular weight for the resulting potato starch modified samples versus the amount of hydrochloric acid used for treating the potato starch is set forth in FIG. 3.

As is shown, the results generally follow the trend exhibited with the high amylose corn starch as discussed in Example 1. Thus, at a given hydrochloric acid concentration for treating, the resulting molecular weight decreases as the amount of the potato starch suspended in the reaction medium decreases. Similarly, at a given level of potato starch being treated, increasing amounts of the treating acid likewise results in the resulting molecular weight being decreased.

10

EXAMPLE 4

This Example is similar to Example 2, except using potato starch instead of a high amylose corn starch.

As in Example 2, methanol, ethanol, 2-propanol and 15 1-butanol were used as the alcohol reaction media. Potato starch was modified in these alcohols for three days while being maintained at a temperature of 25°C and exposed to various concentrations of hydrochloric acid, while the potato starch level was 0.25 kg suspended in 20 one liter of the respective alcohol. The number average molecular weights are set forth in Table 4:

Table 4

HCL Concentration		Alcohol			
(%w/v)	(mL/L)	MeOH	EtOH	2-PrOH	1-BuOH
0.36	10	196020	59940	14580	11016
0.71	20	126360	38394	10433	9736
1.39	40	87966	28206	8780	5897
2.67	80	63990	23184	7063	5557
5.0	160	31590	13446	6642	3969

25

FIG. 4 shows the results obtained using methanol and ethanol while FIG. 5 shows the results using 2-propanol and 1-butanol.

As was also the case with the high amylose corn starch, the resulting molecular weight of the modified potato starch decreased as the reaction medium varied from methanol, to ethanol, 2-propanol and 1-butanol.

- 5 Further, as can be seen, when using methanol or ethanol, the amount of the potato starch being treated and/or the level of hydrochloric acid being used must be coordinated to avoid unduly high molecular weights in the resulting modified potato starch.

10

EXAMPLE 5

- This Example compares the glycemic responses in a study when the modified high amylose starch and potato starch were ingested as well as the use of glucose for comparison.

The modified high amylose corn starch was made by suspending 10 kg of amylo maize-7 starch (Hylon VII, obtained from National Starch & Chemical Corporation, Bridgewater, NJ) in 10 liters of ethanol. The reaction 20 was started by the addition of one liter of concentrated hydrochloric acid and allowed to go three days (72 hours) at ambient temperature (20-21°C) with occasional stirring (approximately 4 times per day). After three days, the starch was allowed to settle, the supernatant was poured 25 off; and five liters of 70% volume for volume (v/v) ethanol was added and the starch was then filtered. The starch was washed on the filter with 70% ethanol until the filtrate was neutral (requiring about 10 liters of 70% ethanol). The starch was spread out in pans and 30 allowed to air dry 15 hours. The resulting material was modified amylo maize-7 starch and had a number average molecular weight of about 38,000. The modified potato starch had a number average molecular weight of 40,000 and was made by a process identical to that described for 35 amylo maize-7.

In the study, at one week intervals, 6 males and 4 females (25 ± 1 year) ingested the modified starches and

glucose at a level of 75 grams dissolved in a final volume of 360 ml water. These various carbohydrates were administered in a randomized, double-blind fashion, and each subject participated in all 3 trials. A 12-hour 5 fasting forearm venous blood sample was collected before ingestion and at 30, 60, 90, 120 and 180 minutes after ingestion. The subjects were required to record all food and beverages consumed for two days prior to their first trial and then to duplicate this diet for the two days 10 prior to each subsequent trial.

Serum was analyzed for glucose concentration and immediately frozen for further analysis. The glycemic index associated with the two modified starches was evaluated by comparing the area under the blood glucose 15 response curve to the starch obtained after ingestion of dextrose. Blood glucose concentration was analyzed for significant differences among the carbohydrates using a two-way (starch x time) repeated measures ANOVA. Hyperglycemic area was analyzed for differences among 20 carbohydrates using a one-way repeated measures ANOVA. When a significant F ratio was obtained with ANOVA, a Newman-Keul post-hoc test was used to locate significant mean differences. Hyperglycemic area after starch ingestion was expressed as a percentage of the 25 hyperglycemic area obtained after glucose ingestion and was analyzed for significant difference using a paired t-test. Differences among means were evaluated at the p<0.05 level. The results are presented as "mean \pm SE."

Because one subject (a female) had an abnormal 30 glucose-tolerance (the blood glucose remained above 8 mM for 3 hours), her data were excluded from the analysis of results. Pre-ingestion blood glucose concentrations were similar among the three trials (modified potato starch: 5.2 \pm 0.2 mM; glucose: 5.2 \pm 0.2 mM; modified high 35 amylose starch: 5.1 \pm 0.1 mM) with an intra-subject coefficient of variation that averaged 3.7%.

The results are set forth in Table 5:

Table 5

Time	Glucose	Modified High Amylose Starch	Modified Potato Starch
Pre-ingestion	5.2 ± 0.1	5.1 ± 0.1	5.2 ± 0.2
30 minutes	7.8 ± 0.6	5.4 ± 0.1	5.2 ± 0.3
60 minutes	5.8 ± 0.3	5.3 ± 0.2	5.1 ± 0.1
90 minutes	5.2 ± 0.4	5.3 ± 0.1	5.1 ± 0.2
120 minutes	4.9 ± 0.3	5.1 ± 0.1	5.1 ± 0.2
180 minutes	4.1 ± 0.2	5.0 ± 0.1	5.1 ± 0.2

5

Thirty minutes after ingestion, the blood glucose concentration rose in the glucose and the modified high amylose starch, but not in the modified potato starch.

10 The peak values for blood glucose concentration were reached at 30 minutes for both glucose and modified high amylose starch. Subsequently, the glucose concentration dropped progressively through the remainder of the sampling period for both. As is seen, at 120 and 180 minutes, the blood glucose concentration was 15 significantly below the pre-ingestion level only after glucose ingestion.

The calculation of the hyperglycemic area revealed that the glucose resulted in the greatest area (122 ± 36 mM·min) while both modified high amylose starch (35 ± 12 mM·min) and the modified potato starch (15 ± 7 mM·min) resulted in significantly lower areas. The difference in the area between the modified high amylose corn and potato starches approached significance ($p = 0.08$). When expressed as a percentage of the area obtained after glucose ingestion, the modified potato starch yielded a glycemic index of $14 \pm 7\%$ and the modified high amylose corn starch yielded a glycemic index of $29 \pm 10\%$.

30 The serum insulin concentration for a three hour period after ingestion was also determined, and the results are set forth in Table 6:

Table 6

Time	Glucose	Modified High Amylose Starch	Modified Potato Starch
Pre-ingestion	7.7 ± 1.4	4.6 ± 0.8	6.1 ± 0.6
30 minutes	47.0 ± 11.0	7.5 ± 0.9	7.5 ± 0.8
60 minutes	53.2 ± 12.7	9.2 ± 4.5	6.1 0.7.1
90 minutes	35.9 ± 6.4	6.6 ± 1.2	5.5 ± 0.7
120 minutes	20.7 ± 3.4	5.7 ± 1.2	5.6 ± 0.8
180 minutes	6.7 ± 1.2	5.6 ± 1.3	5.5 ± 0.5

5

EXAMPLE 6

This Example illustrates the metabolic effects of ingesting the modified starches of this invention in humans.

10 Ten subjects received, on separate occasions, each of the test meals (75 g each of modified high amylose corn starch, modified potato starch, glucose and a placebo) thirty minutes before a 60-minute exercise at an intensity of 70% VO₂max. The modified high amylose corn
 15 and potato starches were those described in Example 5.

In addition to the blood sampling and assays for setting glycemic responses, indirect calorimetry was used to assess caloric expenditure, carbohydrate oxidation and fat oxidation during the exercise period.

20 Table 7 sets forth the blood glucose concentrations (mM) thirty minutes before feeding (listed in the Table as -30 min), before exercise (0 min) and after 30 and 60 minutes of exercise at 70% VO₂max:

25

Table 7

Time	Placebo	Glucose	Modified High Amylose Starch	Modified Potato Starch
-30 min	4.6 ± 0.2	4.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.1
0 min	4.5 ± 0.2	7.7 ± 0.7	5.2 ± 0.2	5.1 ± 0.2
30 min	4.5 ± 0.2	4.4 ± 0.4	5.1 ± 0.2	4.8 ± 0.2
60 min	4.5 ± 0.2	4.6 ± 0.2	4.8 ± 0.2	4.7 ± 0.2

During the placebo trial, the blood glucose was not affected by exercise. However, after the glucose 5 feeding, blood glucose concentration rose to 7.7 ± 0.7 mM at the start of exercise and then dropped significantly to 4.4 ± 0.4 mM thirty minutes into the work period. Both of the modified starches resulted in slight, but significant, increases in blood glucose at the start of 10 the exercise. The elevated blood glucose concentration was maintained during exercise in the modified high amylose corn starch trial, but not in the modified potato starch trial.

The muscle glycogen use was increased significantly 15 in the glucose trial (61 ± 8 $\mu\text{mol/g}$) compared with the placebo trial (40 ± 7 $\mu\text{mol/g}$). Glycogen use during the modified high amylose corn starch trial (45 ± 7 $\mu\text{mol/g}$) was not significantly different from the placebo level, while glycogen use during the modified potato starch 20 trial was not significantly different from that resulted from the use of glucose (55 ± 8 $\mu\text{mol/g}$). The respiratory quotient during the trials was significantly greater during the modified high amylose corn starch and glucose trials (0.953 ± 0.007 and 0.959 ± 0.008 , respectively) 25 than resulted in the modified potato starch and placebo trials (0.943 ± 0.008 and 0.944 ± 0.009 , respectively). These data indicate that, while measures of total carbohydrate availability were increased in the modified high amylose corn starch and glucose trials, the lower 30 glycemic response, and perhaps the sustained level of blood glucose during exercise in the modified high amylose corn starch, prevented the usual acceleration of glycogen use as is seen with glucose.

35

EXAMPLE 7

This Example determines the relative digestibility of the modified starches of the present invention.

The modified high amylose corn starch and potato starch used were those described in Example 5.

Digestibility was determined and compared to that of standard commercial corn starch and glucose.

- 5 Thirty-six 5-week old male rats were randomly divided into groups and fed the four carbohydrates at 55 g/100 g diet. The diet was based on the A.I.N.-76 diet with 10 g fructose/100 g diet, and the composition of the diet is set forth in Table 8:

10

Table 8

Ingredient	Grams
casein	20.0
D,L-methionine	0.3
mineral mix (AIN)	3.5
vitamin mix (AIN)	1.0
choline chloride	0.2
corn oil	5.0
fiber	5.0
fructose	10.0
carbohydrate	55.0
TOTAL	100.0

The rats were fed ad libitum until nine weeks of age.

- 15 Body weight, weight gain, and food consumption were recorded weekly. The body weights and weight gain were similar in all groups. Set forth in Table 9 are the average weekly body weight in grams through week 4:

Table 9

Week	Regular Corn Starch	Glucose	Modified Potato Starch	High Amylose Modified Corn Starch
0	119.8±1.0 ^a	125.8±2.1 ^a	123.4±2.6 ^a	121.3±3.0 ^a
1	186.5±2.5 ^b	186.7±2.5 ^b	192.3±3.7 ^b	190.9±3.7 ^b
2	230.7±3.1 ^c	227.6±2.8 ^c	232.6±4.5 ^c	233.6±5.6 ^c
3	275.7±4.9 ^d	266.4±4.6 ^d	272.2±6.9 ^d	276.0±7.2 ^d
4	286.5±5.6 ^e	283.1±5.4 ^e	291.7±5.1 ^e	295.4±7.6 ^e

Values are the means ± S.E.M. n=9 per group. Means with the different letter are significantly different ($P<0.05$). The analysis of variance indicates that week ($P<0.0001$) was significant. The diet and week*diet interaction were not significant.

5 Food consumption was much higher in the potato starch-fed group ($P < 0.001$) compared to consumption with the other groups. Table 10 sets forth the average weekly food consumption in grams through week 4:

Table 10

10

Week	Regular Corn Starch	Glucose	Modified Potato Starch	High Amylose Modified Corn Starch
1	131.8±2.7 ^a	131.6±2.8 ^{ad}	178.1±3.2 ^b	160.6±3.5 ^c
2	125.9±2.1 ^a	127.3±2.4 ^a	177.5±4.7 ^b	160.8±4.6 ^{cf}
3	136.6±2.6 ^{ad}	135.2±2.8 ^d	187.6±8.6 ^b	173.5±4.3 ^f
4	123.7±8.0 ^a	131.5±7.5 ^{ad}	184.2±8.6 ^b	153.2±10.1 ⁱ

Values are the means ± S.E.M. n=9 per group. Means with the different letter are significantly different ($P<0.05$). The analysis of variance indicates that week ($P<0.0015$), diet ($P<0.0001$) were significant. The week*diet interaction were not significant.

15 The heart weight percent, percent heart/body weight (B.W.), epididymal fat pad weight, and percent fat pad/B.W. were low in the modified potato starch-fed group compared to that of the two control groups. In the high amylose modified corn starch-fed group, only the epididymal fat pad and percent fat pad/B.W. were low. Liver weight percent and percent liver weight/B.W. were 20 higher in both the modified potato starch and high amylose modified corn starch-fed groups. Table 11 sets

forth the organ weights in grams and the percent of organ weight/body weight:

Table 11

Carbohydrate	Organ Weights	(Organ wt./Body wt.) x 100
Heart		
Regular corn starch	1.13±0.04 ^a	0.41±0.01 ^a
Glucose	1.10±0.06 ^a	0.40±0.02 ^a
Modified potato starch	0.97±0.03 ^b	0.35±0.01 ^b
High amylose modified corn starch	1.07±0.04 ^{ab}	0.39±0.01 ^{ab}
Epididymal Fat Pad		
Regular corn starch	2.26±0.16 ^a	0.82±0.04 ^a
Glucose	2.30±0.15 ^a	0.85±0.05 ^a
Modified potato starch	1.54±0.05 ^c	0.57±0.03 ^b
High amylose modified corn starch	1.95±0.14 ^{ac}	0.70±0.04 ^d
Liver		
Regular corn starch	7.77±0.27 ^a	2.83±0.05 ^a
Glucose	7.31±0.23 ^a	2.70±0.05 ^a
Modified potato starch	8.87±0.31 ^b	3.25±0.07 ^b
High amylose modified corn starch	8.94±0.34 ^b	3.24±0.13 ^b
Values are the means ± S.E.M. n=9 for each group. Means with the different letter are significantly different (P<0.05). The analysis of variance indicates that the effect of diet (P<0.0001) was significant for the heart wt., heart wt./body wt., fat pad wt., fat pad wt./body wt., liver wt., and liver wt./body wt.		

The mean liver contents of the rats are set forth in Table 12, the weights set forth being in grams:

Table 12

	Regular Corn Starch	Glucose	Modified Corn Starch	High Amylose Modified Corn Starch
Liver Wt.	7.77±0.27 ^a	7.31±0.23 ^a	8.87±0.31 ^b	8.94±0.34 ^b
% Liver Wt./B. Wt.	2.83±0.05 ^a	2.70±0.05 ^a	3.25±0.07 ^b	3.24±0.13 ^b
Water Content	5.62±0.19 ^a	5.32±0.19 ^a	6.44±0.25 ^b	6.54±0.26 ^b
% Water/Liver Wt.	72.40±0.62 ^a	72.71±0.46 ^a	72.57±0.58 ^a	73.16±0.45 ^a
Total Protein	1.38±0.07 ^a	1.13±0.06 ^b	1.32±0.06 ^{ab}	1.33±0.07 ^a
% Protein/Liver Wt.	17.73±0.65 ^a	15.66±1.01 ^b	14.85±0.53 ^b	14.87±0.57 ^b
Total Lipids	0.32±0.01 ^a	0.32±0.01 ^a	0.43±0.02 ^b	0.38±0.01 ^c
% Lipids/Liver Wt.	4.14±0.09 ^a	4.42±0.11 ^a	4.85±0.16 ^b	4.27±0.16 ^a

Values are the means ± S.E.M. n=9 per group. Means in each row with the different letter are significantly different at the 0.05 level (ANOVA).

5 Table 13 sets forth the mean fasting serum levels of the rats:

Table 13

	Regular Corn Starch	Glucose	Modified Potato Starch	High Amylose Modified Corn Starch
Glucose (mg/dl)	71.9 ± 4.8 ^a	77.1 ± 9.2 ^a	116.5 ± 10.5 ^b	130.9 ± 16.4 ^b
FFA (mM)	0.84± 0.06 ^a	0.92± 0.04	1.18± 0.09 ^b	1.00± 0.33 ^{ab}
TAG (mg/dl)	141.6 ± 8.7 ^a	136.9 ± 10.2 ^a	137.9 ± 9.6 ^a	151.4 ± 11.4 ^a
Protein (g/dl)	7.38± 0.14 ^a	7.06± 0.18 ^a	6.60± 0.15 ^b	7.28± 0.13 ^a
Insulin (μIU/ml)	9.77± 0.99 ^a	9.40± 0.60 ^a	7.15± 0.7 ^b	10.41± 0.52 ^a
Glucagon (pg/ml)	331.5 ±36.3 ^{ab}	304.0 ±33.7 ^b	416.9 ±35.2 ^{ac}	463.6 ±45.2 ^c
Insulin/Glucagon	3.13± 0.37 ^{ac}	3.23± 0.24 ^a	1.79± 0.21 ^b	2.42± 0.24 ^b

Values are the means ± S.E.M. n=9 per group. Means in each row with the different letter are significantly different at the 0.05 level (ANOVA).

10 Digestibility was calculated in terms of glucose units as (Intake = Feces)/Intake. Digestibility was determined as an average of three days, and the results are set forth in Table 14, as are the digestible energy levels:

Table 14

Carbohydrate	Digestibility (%) Carbohydrate	Digestible Energy (%) (diet)
Regular corn starch	100.00 ± 0.00 ^a	94.17 ± 0.22 ^a
Glucose	100.00 ± 0.00 ^a	94.23 ± 0.17 ^a
Modified Potato Starch	69.01 ± 1.17 ^b	70.77 ± 0.67 ^b
High Amylose Modified Corn Starch	91.54 ± 0.83 ^c	83.62 ± 1.14 ^c
Values are the means ± S.E.M. n=9 per group. Means with the different letter are significantly different (P<0.005). The analysis of variance indicates that the diet (P<0.0001) was significant.		

EXAMPLE 8

- 5 This Example shows the effect upon the processing and pasting profile properties due to acid treating various starches, food grade waxy maize (WM), maize (M) amylo maize-5 (Amy5), and amylol maize (Amy 7) were analyzed in both their unmodified and modified forms.
- 10 Modification of these starches was performed by suspending 0.25 kg of starch in 1 liter 100% ethanol. The reaction was started by adding concentrated hydrochloric acid to this mixture at a final concentration of 0.36%. The reaction continued for 3
- 15 days 20-22° C. The starches were then filtered and washed and repeated rinsing with 70% ethanol. Starches were then dried at room temperature for a minimum of 5 days before analysis.

Table 15 shows the results of the DSC analyses of
20 the unmodified and modified starches tested:

Table 15

	Peak Onset (°C)	Enthalpy 1 (J/g)	Peak Onset 2 (°C)	Enthalpy 2 (J/g)
WV-unmod.	64.8	14.1	42.1	3.3
WV-mod.	62.3	13.5	45.7	6.0
M-unmod.	64.9	11.3	41.6	5.7
M-mod.	61.5	11.5	42.6	5.3
Amy5-unmod.	70.9	14.8	81.2	7.1
Amy5-mod.	66.0	13.1	61.2	8.0
Amy7-unmod.	68.0	14.0	85.3	3.7
Amy7-mod.	67.3	11.3	74.4	5.1

The temperature at which the starches began to gel is Peak Onset 1. As can be seen, the modified starches uniformly began to gel at temperatures about 1-3° C. lower than the unmodified starches.

5 Enthalpy 1 reflects the amount of energy required to produce the gel and, with the exception of ordinary maize (M), is lower in the modified starches compared to the unmodified starches. This effect is particularly striking in the amylo maize-7 starch in which modification 10 decreased enthalpy by 19%.

Peak Onset 2 and Enthalpy 2 were measured after the starches were held for 7 days and allowed to retrograde. After retrogradation, modification had no appreciable effect on peak onset in either waxy maize or ordinary maize. Peak onset after retrogradation was markedly reduced in the modified high amylose starches (Amy5 and Amy7) compared to their unmodified forms.

Results from the pasting analysis are set forth in Table 16:

20

Table 16

	PV (rvu)	HPV (rvu)	Breakdown (rvu)	CPV (rvu)	Setback (rvu)	Pasting Temp. (°C)
Waxy						
Unmod.	204.3	84.5	119.8	107.6	23.1	72.1
Mod.	2.2	0.6	1.6	1.0	0.4	n/a
Maize						
Unmod.	106.8	77.5	29.3	142.7	65.2	85.6
Mod.	8.2	1.0	7.1	3.1	2.1	n/a
Amy5						
Unmod.	2.6	1.6	1.1	1.4	0.2	n/a
Mod.	2.1	0.8	1.3	1.3	0.5	n/a
Amy7						
Unmod.	1.2	0.0	1.2	0.1	0.1	n/a
Mod	6.8	-6.2	12.3	-6.0	0.1	n/a

All measures of viscosity including peak viscosity (PV), hot paste viscosity (HPV), breakdown, cool paste viscosity (CPV), and setback were reduced by modification in both waxy maize and ordinary maize. The same effect was not observed in the amylo maize-5 (Amy5) or amylo maize-7 (Amy7) due to the fact that even the 30 unmodified forms of these starches do not paste at the

temperatures reached by this equipment. Stated differently, the equipment used was not appropriate to determine the effect upon the pasting profiles due to acid treatment. Accordingly, the Amy5 and Amy7 results
5 are only included for completeness.

Thus, as has been seen, the present invention provides modified starch materials that have desirable physiological responses, permitting their desirable use
10 in a variety of nutritional products. The use of modified corn starch, particularly amylo maize-7, provides particular advantage, both due to the enhanced digestibility as well as a relatively slow release of metabolizable carbohydrates, giving a source of
15 carbohydrate energy over a much longer period of time than can be obtained from glucose and other carbohydrates such as lactose, fructose, or sucrose. The ability to further alter the processing and pasting profiles provides the opportunity to tailor the modified starches
20 to the desired processing and pasting profile requirements of specific applications.

WE CLAIM:

1. A modified starch comprising an acid-treated starch having a number average molecular weight of at least about 10,000 and a Peak Onset temperature of at least 1° C. lower and an Enthalpy of about 1 to 50% less than that of the unmodified starch.

2. The modified starch of claim 1 wherein said starch is amylo maize-5.

3. The modified starch of claim 1 wherein said starch is amylo maize-7.

4. A modified starch comprising an acid-treated starch having a number average molecular weight of at least about 10,000, said modified starch being retrograded and having a Peak Onset 2 of at least 5°C. less than that of the unmodified, retrograded starch.

5. The modified starch of claim 4 wherein Peak Onset 2 is at least 10° C. less than that of the unmodified, retrograded starch.

6. The modified starch of claim 4 wherein said starch is amylo maize-5.

7. The modified starch of claim 4 wherein said starch is amylo maize-7.

8. A modified starch comprising an acid-treated starch having a number average molecular weight of at least about 10,000 and having at least one pasting profile characteristic reduced by at least 50% relative to that of the unmodified starch.

9. The modified starch of claim 8 wherein said pasting profile characteristic is peak viscosity.

10. The modified starch of claim 8 wherein, said pasting profile characteristic is hot paste viscosity.

11. The modified starch of claim 8 wherein said pasting profile characteristic is the breakdown of starch paste upon continued heating after obtaining paste viscosity.

12. The modified starch of claim 8 wherein said pasting profile characteristic is the viscosity of starch paste upon cooling after heating.

13. The modified starch of claim 8 wherein said pasting profile characteristic is the setback of the starch paste.

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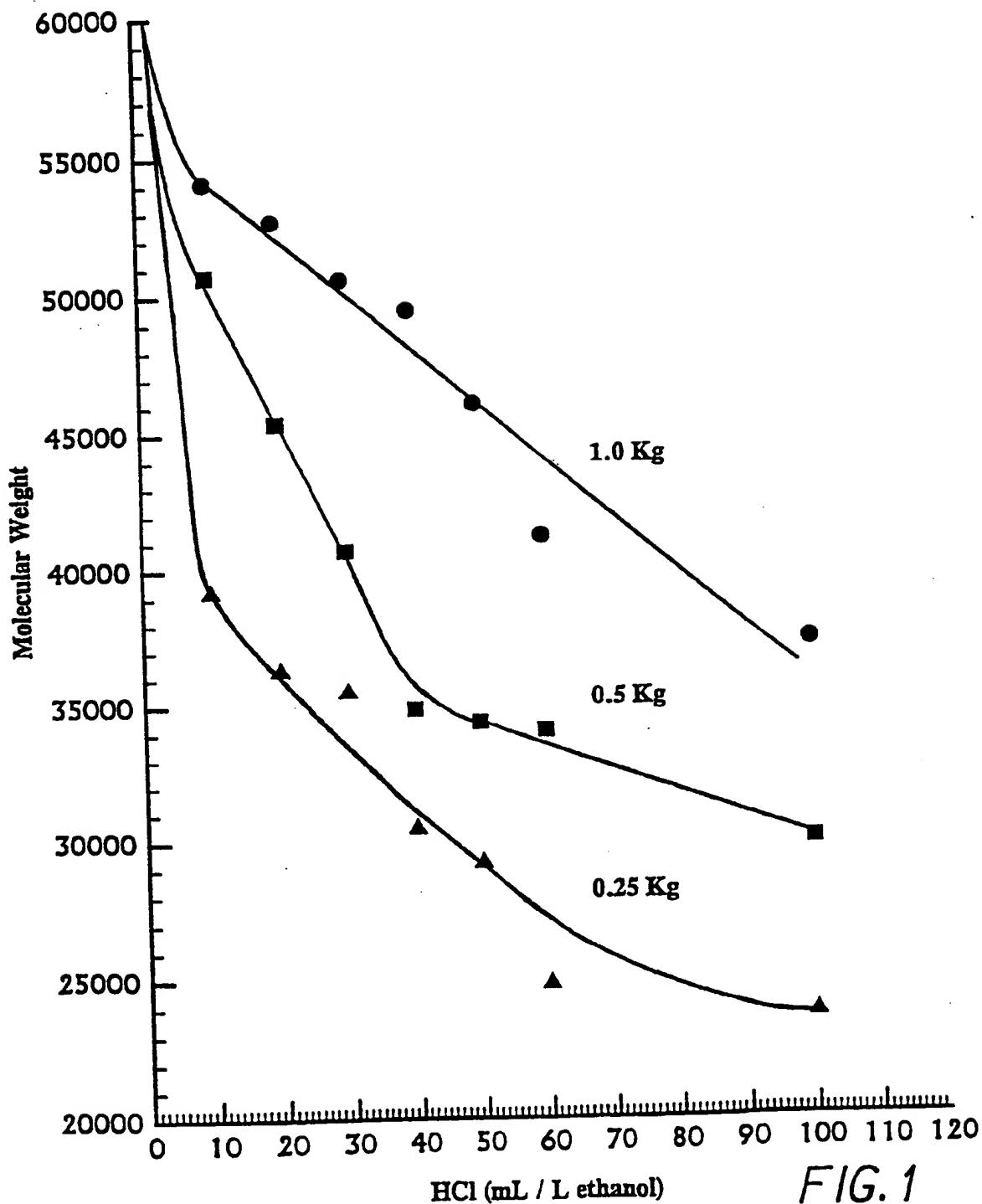


FIG. 1

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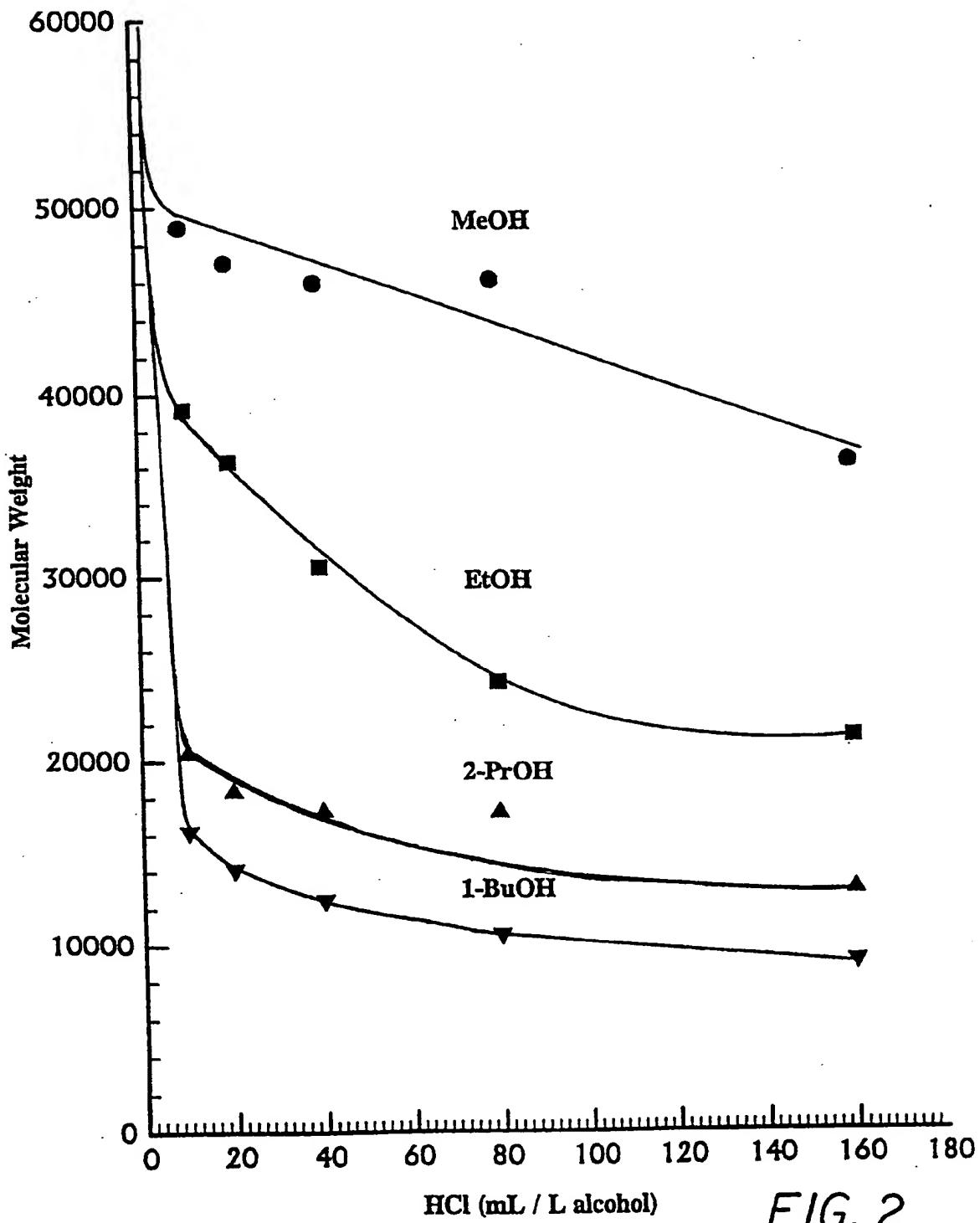


FIG. 2

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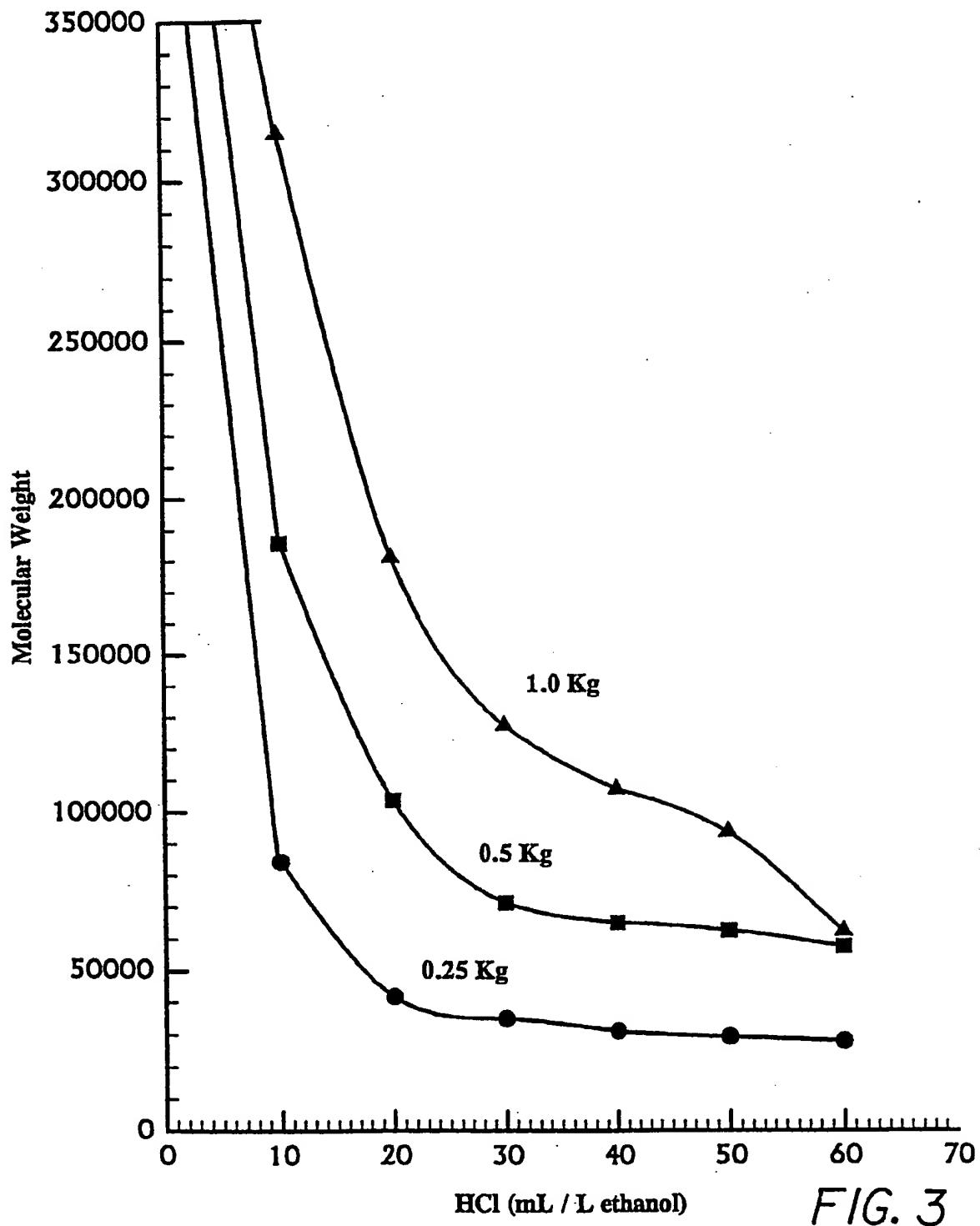


FIG. 3

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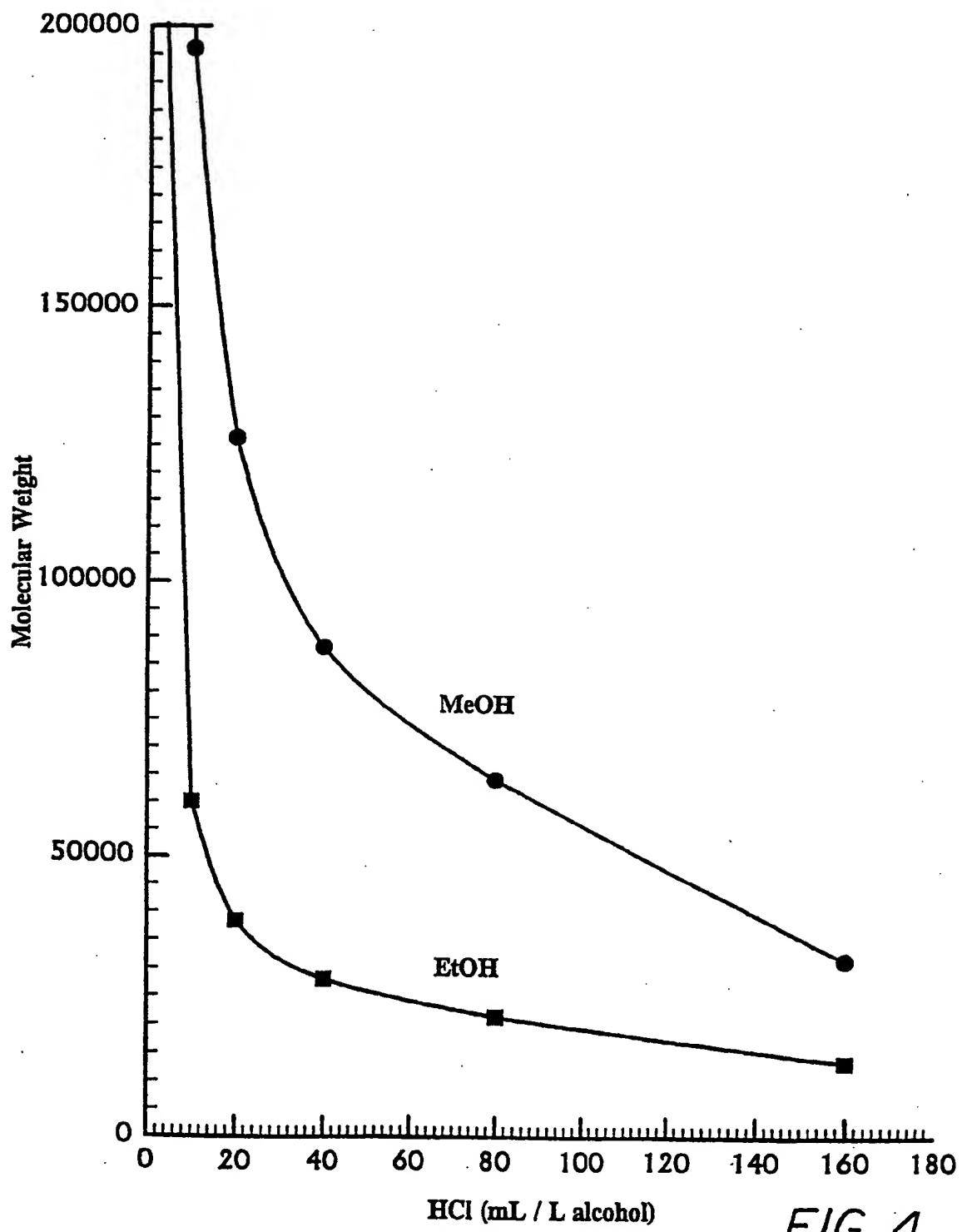


FIG. 4

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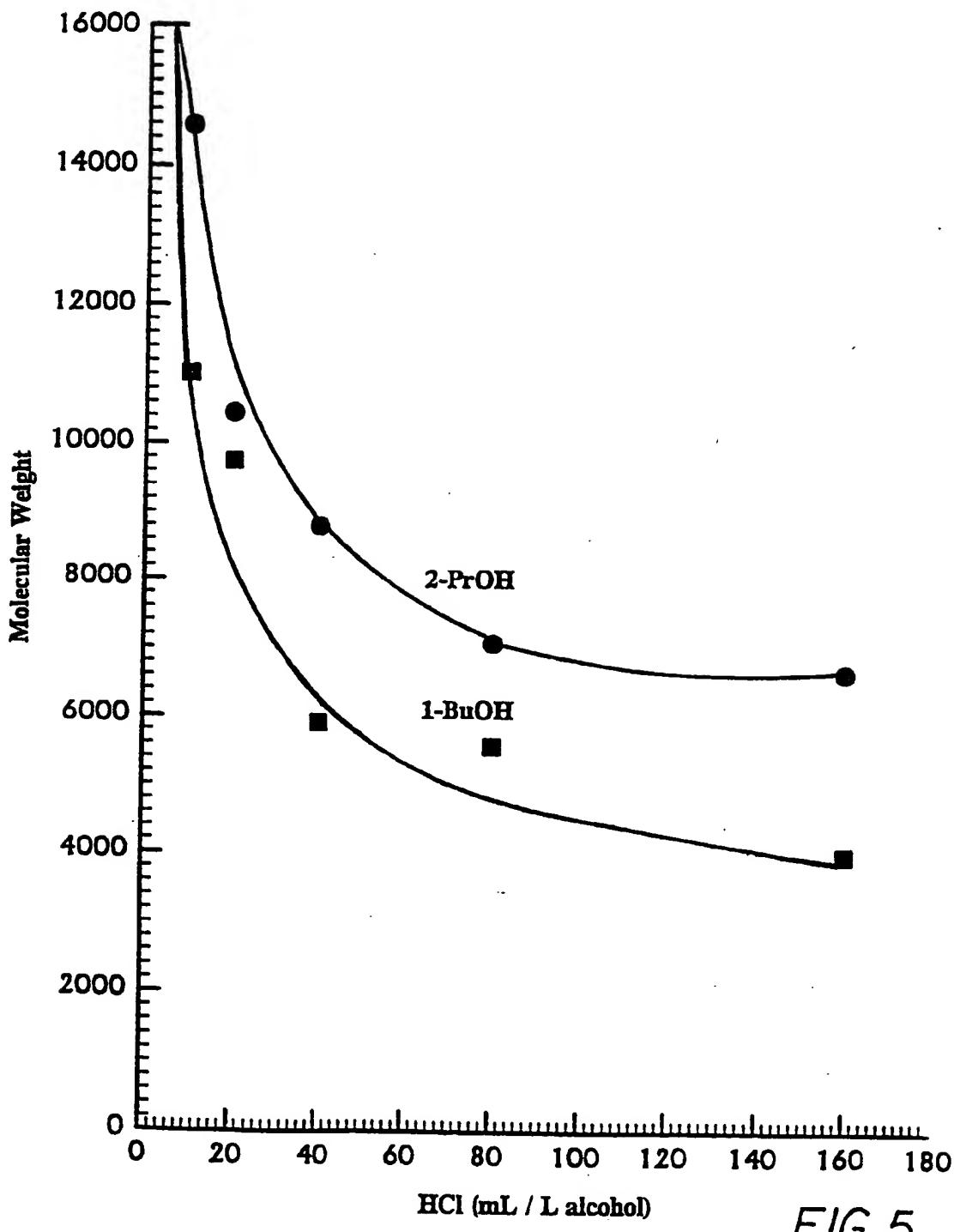


FIG. 5

INTERNATIONAL SEARCH REPORT

Inten. Appl. No
PCT/US 99/22664

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L1/0522 A23L1/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 695 803 A (KAPLAN MURRAY L ET AL) 9 December 1997 (1997-12-09) cited in the application see the whole document	1-13
X	WO 94 18850 A (STALEY MFG CO A E) 1 September 1994 (1994-09-01) examples 1,2	1-13
X	WO 94 05163 A (STALEY MFG CO A E) 17 March 1994 (1994-03-17) examples	1-13
X	WO 94 09645 A (STALEY MFG CO A E) 11 May 1994 (1994-05-11) examples 1,2	1-13
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the International search

25 February 2000

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/22664

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE FSTA 'Online! INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE AN 94-1-03-10027, 1994 "Properties of ligninized waxy rice starches" XP002131573 abstract & YANG-KYUN PARK ET AL.: JOURNAL OF THE KOREAN SOCIETY OF FOOD AND NUTRITION, vol. 22, no. 5, - 1993 pages 596-602,	1-13
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat...ional Application No

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